PRODUCT INFORMATION & MANUAL

Glucose Oxidase Activity Assay Kit (Colorimetric)

NBP3-24491

For research use only.
Not for diagnostic or therapeutic procedures.

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Glucose Oxidase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24491

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.006 U/L

Detection range: 0.006–0.1 U/L

Average intra-assay CV (%): 3

Average inter-assay CV (%): 7

Average recovery rate (%): 101

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure glucose oxidase (GOD) activity in samples.

▲Detection principle

Glucose oxidase is an important industrial enzyme in the food industry, widely used in wine, beer, juice, milk powder and other food deoxygenation, flour improvement, food browning prevention and other aspects, in food rapid detection and biosensors are also widely used. Microorganisms are the main source of GOD production with rapid growth and reproduction and wide sources, and the main production strains are aspergillus niger and penicillium.

Glucose oxidase can catalyze the oxidation of glucose to produce hydrogen peroxide. In the presence of peroxidase, hydrogen oxidizes pigment sources to form colored substances. Measure the OD value at 550 nm and glucose oxidase (GOD) activity can be calculated indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	-20℃ , 12 months
Reagent 2	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 3	Chromogenic Agent	1.6 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 4	1 mmol/L Standard	1.6 mL × 2 vials	-20℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

MicroPipettor, Water bath, Centrifuge, Microplate reader (540-560 nm), Incubator, 10 KD Ultrafiltration tube

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dissolve a vial of powder with 0.2 mL double distilled water. Prepare fresh solution before use and can be stored at -20°C for 1 week. Avoid to repeated freeze-thawing.
3. The preparation of reaction working solution:
   Mix the reagent 1 and reagent 2 working solution at a ratio of 49:1. Prepare the fresh solution before use. Preserve the prepared working solution on the ice for use, and should be used up within 4 h.

▲ Sample preparation

Sample:

Accurately weigh the tissue, add 9 times the volume of normal saline (0.9% NaCl) according to the ratio of Weight (g): Volume (mL) =1:9. Homogenize the tissue sample with homogenizer on ice. Centrifuge the homogenized tissue at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.006–0.1 U/L).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 1 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	100
B	0.2	20	80
C	0.3	30	70
D	0.4	40	60
E	0.6	60	40
F	0.8	80	20
G	0.9	90	10
H	1	100	0

2. The measurement of samples

(1) Standard well: Take 10 μL of standard solution with different concentration to the wells.

Sample well: Take 10 μL of sample to the wells.

(2) Add 200 μL of reaction working solution into the each well.

(3) Add 20 μL of reagent 3 into each well.

(4) Mix fully for 3 s with microplate reader. Measure the OD value of each well with microplate reader at 550 nm, recorded as A_1.

(5) Incubate at 37°C for 20 min.

(6) Measure the OD value of each well with microplate reader at 550 nm, recorded as A_2, $\Delta A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of $A_{2(standard)}$).

▲Summary operation table

	Standard well	Sample well
Standard with different concentration (μL)	10	
Sample (μL)		10
Reaction working solution (μL)	200	200
Reagent 3 (μL)	20	20
Mix fully. Measure the OD value of each well, recorded as A_1.		
Incubate at 37°C for 20 min. Mix fully. Measure the OD value of each well, recorded as A_2, $\Delta A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of $A_{2(standard)}$).		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

Definition : The enzyme amount of 1 μmol of hydrogen generated by 1 g sample protein per minute at 37°C is defined as 1 unit.

$$\text{GOD activity (U/gprot)} = (\Delta A_{550} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0, standard only detect A_2 value).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{550}: ΔA_{Sample} (A_2–A_1).

T: Reaction time, 20 min

1000: 1 mmol/L=1000 μmol/L

C_{pr}: Concentration of protein in sample, gprot/L

f: Dilution factor of sample before test.